EFFECT OF JUICE EXTRACTOR SETTINGS ON HAMLIN ORANGE JUICE CLOUD STABILITY[1]

RANDALL G. CAMERON[2], ROBERT A. BAKER, BÉLA S. BUSLIG and
KAREL GROHMANN

USDA, ARS, SAA
Citrus and Subtropical Products Laboratory
P.O. Box 1909
Winter Haven, FL 33883

ABSTRACT

Three different juice extractor settings were used to obtain juice from Hamlin oranges. Cloud in Soft and Medium extracted juice had nearly identical A_{660} values. The A_{660} of the Hard extracted juice was greater than the others. At 30C A_{660} for both the Soft and Medium juice increased slightly over time while the Hard juice A_{660} decreased. The cloud in all three raw juices showed a decrease in A_{660} at 4C. Specific activity of pectinmethylesterase in the raw juices was very similar, but was higher in protein extracts from the Hard juice than from Medium or Soft juices. Addition of protein extracts from each juice, to reconstituted FCOJ, demonstrated that cloud loss in these treatments was dependent on extraction pressure. At 30C PME in the extract from the Soft extracted juice destabilized the cloud most rapidly. Cloud loss also was dependent on the amount of PME activity added.

INTRODUCTION

Processing of sweet oranges for production of Frozen Concentrated Orange Juice (FCOJ) and Not-From-Concentrate juice utilizes the vast majority of sweet oranges harvested in the United States (Florida Citrus Mutual 1999). Several

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[2] Correspondence should be sent to: Dr. Randall G. Cameron, USDA, ARS, SAA, Citrus and Subtropical Products Laboratory, P.O. Box 1909, Winter Haven, FL 33883. Phone: 863-293-4133, ext. 124; Fax: 863-299-8678; Email: rcameron@citrus.usda.gov

varieties of oranges are grown in Florida, with different varieties maturing at different times during the harvesting season (Swift and Veldhuis 1957). The major early season variety of sweet oranges in Florida is *Citrus sinensis* cv. Hamlin. Several characteristics used to describe juice differ between Hamlin juice and juice from mid- and late-season varieties, i.e. Pineapple and Valencia (Bitters 1961; Attaway *et al.* 1972; Carter *et al.* 1975; Fellers *et al.* 1975; Huggart *et al.* 1975; Stewart 1977; Kesterson 1980). Juice from Hamlin oranges had less cloud (as measured by light transmission) and paler color than Pineapple or Valencia orange juice (Attaway *et al.* 1972; Huggart *et al.* 1975). The poor color and weak flavor of early Hamlin juice necessitate blending with juice from other varieties (Saunt 1990). As an early season fruit the Hamlin harvest is less severely affected by freezing weather and is, therefore, of relatively low risk to the grower (Attaway 1997).

Although there has been considerable characterization of various components and qualities of Hamlin juice cloud (see references above) there have been no reports, to our knowledge, on cloud stability of Hamlin juice or the effects of juice extractor settings on Hamlin juice cloud stability. Attaway *et al.* (1972) investigated the effects of extractor settings on numerous juice parameters, including cloud (as % light transmission), but did not ascertain the dynamic effects on cloud stability or amount of PME included in the juice. Nor did Attaway *et al.* (1972) determine if the mixture of PME forms from different extraction pressures had differential effects on cloud stability of previously pasteurized juice. Juice cloud stability is related to the activity of the enzyme pectinmethylesterase (PME, EC 3.1.1.11; see Guyer *et al.* 1956; Bissett *et al.* 1957 and Carroll *et al.* 1957 for discussions and a definition of clarification). Cloud loss results in a clear, practically flavorless serum and the sedimented solids. Sequential hydrolysis of C-6 methyl esters in the homogalacturonan region of soluble pectin by PME produces a stretch of demethylated galacturonic acid residues. These pectins can then be cross-linked with calcium or other divalent cations (Baker and Bruemmer 1969; Baker 1979). The increase in apparent molecular weight, due to cross-linking, reduces solubility and leads to flocculation. While there is some evidence that PME acts on pectins at the surface of cloud particulates (Yufera *et al.* 1965) and that cloud particulates may bind to flocculating pectins (Baker and Bruemmer 1972), it is believed that it is the increased cross-linking of pectins and the resulting occluded particulates that removes them from suspension (Stevens *et al.* 1950; Joslyn and Pilnik 1961). Gelation of FCOJ can also occur due to higher pectin concentrations (Olsen *et al.* 1951), ranging from curdiness to firm gel formation. Gelation of FCOJ hinders reconstitution to single strength juice and may interfere with handling. Citrus juices are commonly pasteurized at a temperature range of 90 to 95C for 15-60 s (Chen *et al.* 1993) to inactivate PME and eliminate these product defects.

We have demonstrated that juice extractor pressure does affect juice cloud stability in Valencia orange juice (Cameron *et al.* 1999), the major late-season variety. Additionally, PME present in protein extracts from the expressed juices destabilized the cloud of reconstituted, pasteurized FCOJ at differential rates. PME from Hard Extracted Juice destabilized the cloud the most rapidly, followed by PME from Medium Extracted Juice and, finally, Soft Extracted Juice when the PME activity in the protein extracts was estimated at pH 4.5. We also have previously demonstrated that PME from Valencia peel tissue destabilized juice cloud more rapidly than PME from rag tissue (intersegmental septa, squeezed juice sacs and fruit core tissue) or hand-expressed juice (Cameron *et al.* 1997). Two of the four PME isoforms partially purified from Valencia peel extracts, one thermally tolerant and the other thermally sensitive, rapidly precipitated the juice cloud in the reconstituted, pasteurized FCOJ (Cameron *et al.* 1998).

This study was designed to test the effect of juice extractor settings on cloud stability of raw Hamlin orange juice and the effects of total salt extractable proteins, from each different juice extraction, on the juice cloud stability of reconstituted, pasteurized FCOJ.

MATERIALS AND METHODS

Methods used for this study were previously described by Cameron *et al.* (1999). A brief description follows.

Juice Extraction

Field run Hamlin oranges (early season, November 3, 1997) were purchased from a local growers association (Haines City Citrus Growers Association, Haines City, FL). The fruit was washed and juice was extracted with an FMC Model 291 Juice Extractor equipped with 3 in. fruit cups and an FMC Model 35 Finisher (0.40 in.). A 5/8 in. long bore orifice tube (0.040 in. strainer) and 1/8 in. beam was used for the 'Soft' extraction (Soft Extracted Juice = SEJ), 7/16 in. long bore orifice tube (0.040 in. strainer) and 3/4 in. beam for the 'Medium' extraction (Medium Extracted Juice = MEJ) and a 7/16 in. long window tube with 1/8 in. beam was used for the 'Hard' extraction (Hard Extracted Juice = HEJ). Settings for 'Hard' and 'Soft' extractions are identical to those described by Attaway *et al.* (1972). Settings for the 'Medium' juice extraction were recommended to provide an intermediate condition between the 'Hard' and 'Soft' extraction. Briefly, increasing the beam size allows the two halves of the fruit support cup to come closer together and use of a window tube eliminates the 'prefinisher' effects of the orifice (strainer) tube, allowing more

of the internal fruit cellular material to pass into the juice manifold (see Shaw and Nagy 1993 for a detailed description of the operation of an FMC juice extractor).

Pectinmethylesterase Extraction

Total salt extractable proteins were obtained from 16 L of each juice sample (Cameron *et al.* 1997). Briefly, juice was brought to 0.1 M Tris Base, 1.0 M NaCl and then adjusted to pH 8.0 with solid NaOH. This solution was stirred overnight at 5C. The following morning the juice was filtered through 4 layers of cheesecloth and then centrifuged at 12,000 × g for 30 min at 4C. The supernatant was decanted, pooled and then brought to 75% saturation with solid ammonium sulfate, stirred overnight at 5C and then centrifuged as described above. The resulting pellets were solubilized in 10 mM Tris, pH 7.5 (at 31 C), 20 mM NaCl and 0.02% sodium azide (w/v). The solubilized protein was exhaustively dialyzed (6000 Dalton molecular weight cut-off dialysis tubing) at 5C against 4 L solubilization buffer, with a total of 4 buffer changes. A precipitate that formed during dialysis was removed by centrifugation as previously described.

Enzyme Activity Assays

Pectinmethylesterase activity in the raw juice was estimated according to Cameron and Grohmann (1996) using a kinetic microplate method (Cameron *et al.* 1992) at pH 7.5 (n = 3) and 0.05% (w/v) citrus pectin (Degree of Methylation = 72%, Sigma). Activity of PME in protein extracts also was estimated at pH 4.5 (n = 15) by adapting the colorimetric method of Vilarino *et al.* (1993) to a kinetic microplate reader. All solutions were adjusted to pH 4.5 just prior to use. The final reaction mixture contained 0.05% (w/v) citrus pectin, 154.5 μg · mL^{-1} bromocresol green and 0.2 M NaCl.

Juice Cloud Stability

Raw juice was brought to 0.02% (w/v) sodium azide and 1.74 g · L^{-1} potassium metabisulphite was added. Juice samples were stored in glass bottles at both 4 and 30C. At selected times the juice was sampled and juice cloud stability was determined as previously described (Cameron *et al.* 1997) by monitoring absorbance at 660 nm.

Pasteurized FCOJ was obtained from a local processor (Florida's Natural Growers, Lake Wales, FL) and reconstituted to 11°Brix with deionized water plus one of the PME containing extracts from either the SEJ (Soft PME), MEJ (Medium PME) or HEJ (Hard PME). Concentrations of 0.05, 0.1 and 0.25 Unit · mL^{-1} PME (estimated at pH 4.5, one Unit is the volume required to release

1 μEq $\cdot$ min^{-1}) were added to the juice. Preliminary experiments indicated that addition of 1.0 Unit $\cdot$ mL^{-1} destabilized the juice cloud too rapidly to be able to ascertain differences between the treatments. In addition to the PME containing extract the reconstituted FCOJ was made to contain 0.02% (w/v) sodium azide and 1.74 g $\cdot$ L^{-1} potassium metabisulphite. At selected times juice was sampled and juice cloud stability was determined by monitoring absorbance at 660 nm (Krop 1974). Briefly, three samples per treatment were taken from each bottle per time interval by inverting the bottle three times, pipetting 10 mL into a 15 mL graduated, conical centrifuge tube, and then centrifuging for 10 min at 360 $\times$ g. One mL of supernatant from each tube was transferred to a cuvette and the absorption at 660 nm was recorded. Krop (1974) adopted 660 nm to measure light absorption in part because turbidity measurements were widely used to determine cloud stability, 650 nm and 660 nm were the most frequently used wavelengths and wavelengths below 600 nm resulted in attenuation of the light beam by absorption of yellow color of the juice.

RESULTS

The cloud of raw Hamlin juice was more stable at 30C than 4C (Fig. 1). At 30C only the HEJ had a decrease in A_{660} over the three week study period. Although the HEJ did contain more suspended solids than the SEJ or MEJ, as evidenced by the greater initial A_{660} of the HEJ (Fig. 1), its initial A_{660} of approximately 1.0 is a low value. Both the SEJ and MEJ cloud A_{660} remained nearly constant throughout the study period. At 4C the A_{660} of all the samples decreased through time (Fig. 1). The greatest reduction was in the HEJ. Changes in A_{660} at 4C of both MEJ and SEJ were nearly identical. Only minor changes were observed for the settling pulp of these samples (Fig. 2). The HEJ had the greatest amount of settling pulp and the SEJ had the least. Although A_{660} values for the SEJ and MEJ were very similar the amount of settling pulp in these samples differed by several percentage points. Again, for all treatments, there was more settling pulp when incubated at 4 vs 30C (Fig. 2).

The amount of total salt extractable protein present in the raw juice was lowest in the SEJ and highest in the HEJ (Table 1). PME activity also was the highest in the HEJ and lowest in the SEJ (Table 1). However, based on specific activity (μEq $\cdot$ min^{-1} $\cdot$ mg^{-1} protein), the differences were minimal at pH 4.5, which is near the pH of juice ($\sim$3.9). All three protein extracts demonstrated nearly equal reductions in PME activity when estimated at pH 4.5 vs pH 7.5 (Table 1). Based on specific activity at pH 7.5, PME from HEJ had higher levels of PME per unit protein than the other treatments.
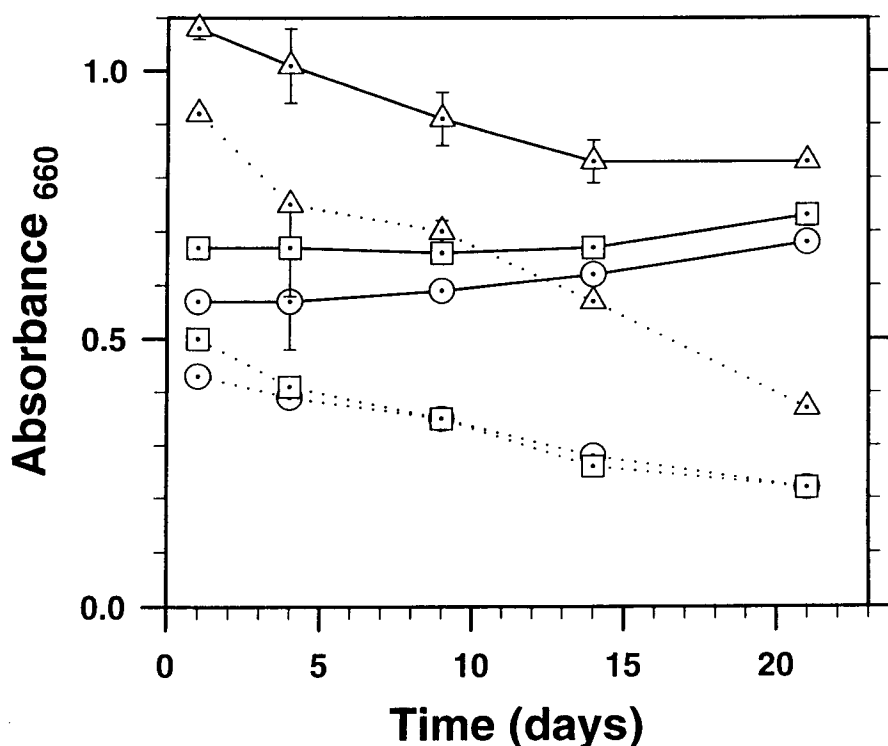
FIG. 1. CLOUD STABILITY OF RAW HAMLIN JUICE
— = 30C incubation, ----- = 4C incubation, O = Soft Extracted Juice,
□ = Medium Extracted Juice, △ = Hard Extracted Juice.

Juice cloud of reconstituted, pasteurized FCOJ was destabilized by the addition of PME containing protein extracts from the different juice treatments when incubated at 30C (Fig. 3). The rate of cloud destabilization was dependent on the amount of PME activity added. Over a six week period only very small decreases were observed in the A_{660} when 0.05 Unit $\cdot$ mL^{-1} was added to the juice. Two separate trials with the addition of 0.10 Unit $\cdot$ mL^{-1} produced nearly identical decreases in A_{660}. The 0.05 Unit $\cdot$ mL^{-1} and 0.25 Unit $\cdot$ mL^{-1} trials were not replicated because they were either too slow or too fast, respectively, to provide reliable data. In both cases the PME from the SEJ caused the most rapid cloud destabilization. The PME from the HEJ had the least effect on cloud stability. Settling pulp in this sample increased concomitantly with the decrease

in A_{660} (Fig. 4). The addition of 0.25 Unit $\cdot$ mL^{-1} caused much more rapid cloud loss, but the SEJ still produced the most rapid effect. At 4C the addition of 0.25 Unit $\cdot$ mL^{-1} had no effect over a six day period (Fig. 5). At 30C this same level of SEJ PME activity had clarified the juice cloud, and the MEJ and HEJ PME had reduced the A_{660} by nearly 50% (compare to Fig. 3).
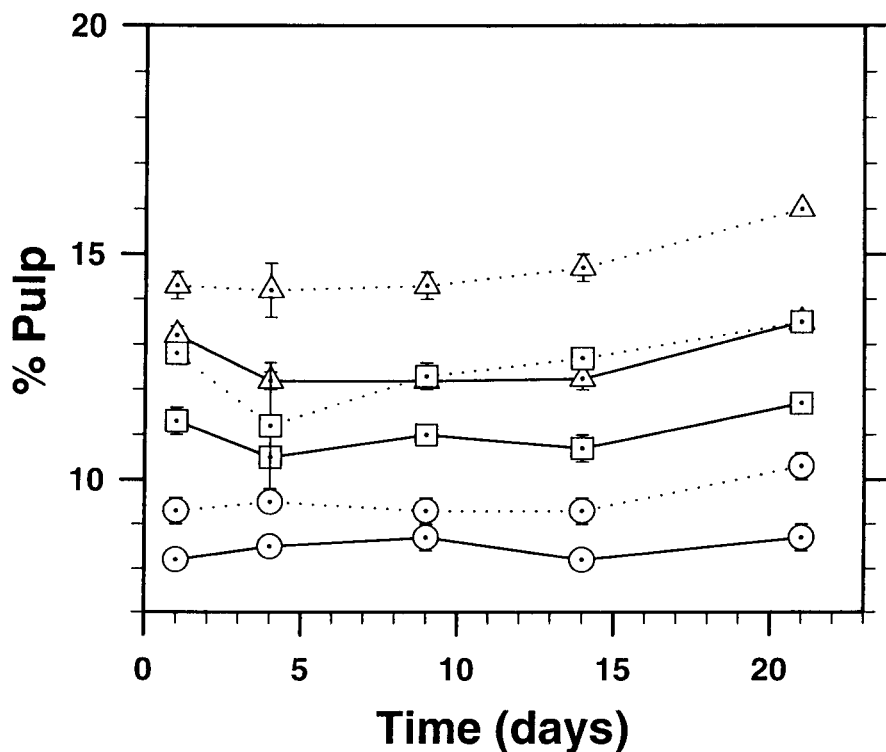

FIG. 2. SETTLING PULP IN RAW HAMLIN JUICE
Symbols and lines as in Fig. 1.

TABLE 1.
PROTEIN AMOUNTS AND PECTINMETHYLESTERASE ACTIVITY IN
RAW JUICE AND PROTEIN EXTRACTS FROM SOFT, MEDIUM AND HARD JUICE.

Sample	Juice Extraction	Protein $(mg \cdot mL^{-1})$	Activity[a,b]		
			pH 4.5	pH 7.5	pH 4.5/pH 7.5 x 100%[c]
Raw Juice	Soft	0.82 ± 0.02	NM	3.0 ± 0.1[a] 3.7[b]	-----
	Medium	1.04 ± 0.01	NM	4.0 ± 0.1[a] 3.8[b]	-----
	Hard	1.24 ± 0.04	NM	5.0 ± 0.2[a] 4.0[b]	-----
Protein Extracts	Soft	11.0 ± 0.9	22.0 ± 1.0[a] 2.0[b]	113.0 ± 2.0[a] 10.3[b]	19.5%
	Medium	12.7 ± 0.8	27.0 ± 2.0[a] 2.0[b]	120.0 ± 2.0[a] 9.4[b]	22.5%
	Hard	6.9 ± 0.1	24.0 ± 2.0[a] 3.0[b]	117.0 ± 4.0[a] 16.9[b]	20.5%

[a] $\mu Eq \cdot min^{-1} \cdot mL^{-1}$
[b] Specific Activity, $\mu Eq \cdot min^{-1} \cdot mg^{-1}$ protein
[c] based on $\mu Eq \cdot min^{-1} \cdot mL^{-1}$
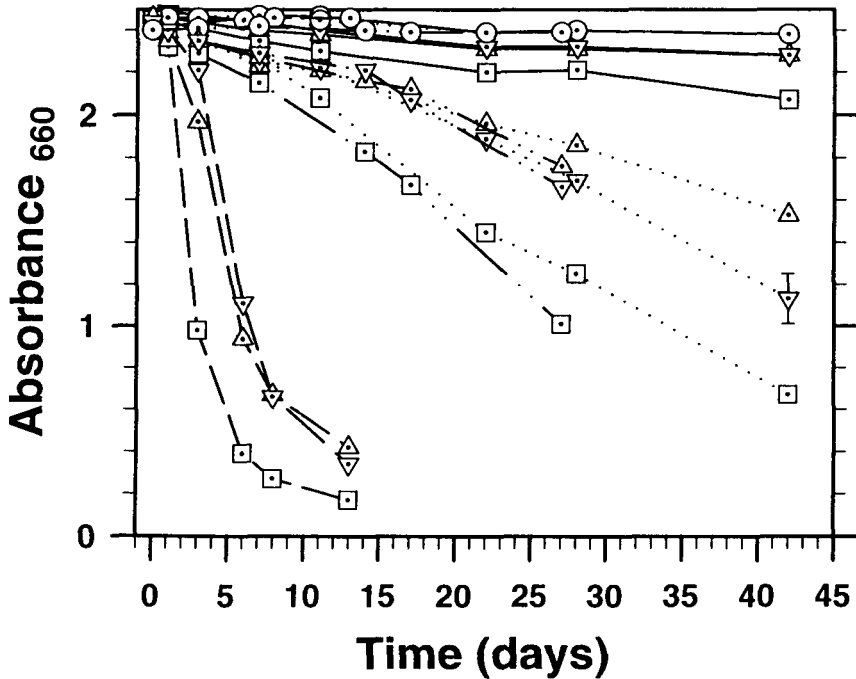NM = Not Measurable

FIG. 3. CLOUD STABILITY AT 30C OF PASTEURIZED, RECONSTITUTED FCOJ TO
WHICH PME CONTAINING PROTEIN EXTRACTS FROM SOFT, MEDIUM OR HARD
JUICE EXTRACTION HAD BEEN ADDED
— = 0.05 Unit · mL^{-1}; ----- = 0.10 Unit · mL^{-1}, Replicate 1; —-— = 0.10 Unit · mL^{-1},
Replicate 2; --- = 0.25 Unit · mL^{-1}; O = Control; □ = Soft; Δ = Medium; ∇ = Hard.

DISCUSSION

Orange juice obtained from Hamlin oranges at different juice extractor
settings had very different juice cloud light absorbing properties and stability
than juice from Valencia oranges (see Cameron *et al.* 1999). The juice cloud
from raw Valencia juice, obtained at the same extractor settings as used here,
had initial A_{660} values two to three times higher than the Hamlin juice. Based on
light transmission properties, Huggart *et al.* (1975) also concluded that Hamlin
juice had less cloud than Valencia juice. Although the Hamlin juice cloud is
paler than Valencia, it appears to be generally more stable. Data presented here
did not demonstrate the large decrease in A_{660} that was observed in raw Valencia
juice (Cameron *et al.* 1999), especially for the HEJ at a 30C incubation. In fact,
at 30C only the HEJ had a decrease in A_{660}. Both the MEJ and SEJ increased
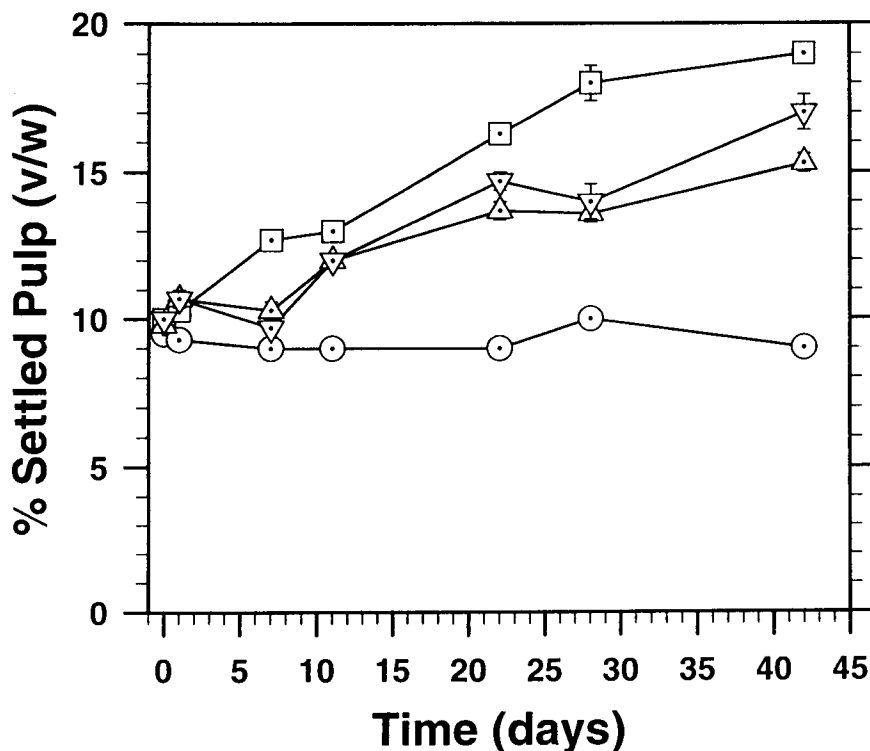
FIG. 4. PERCENT SETTLED PULP IN PASTEURIZED, RECONSTITUTED FCOJ TO
WHICH PME CONTAINING PROTEIN EXTRACTS FROM SOFT, MEDIUM AND HARD
JUICE EXTRACTION HAD BEEN ADDED AT 0.10 UNIT · ML⁻¹ (REPLICATE 1)
AND INCUBATED AT 30C
Symbols as in Fig. 3.

their A_{660} at 30C. The Hamlin samples did have a very large initial difference
in A_{660} between the HEJ and SEJ or MEJ, which was not observed in the
Valencia juice. The HEJ A_{660} was nearly twice as high as the other two
treatments (see Fig. 1). The differences observed for the changes of A_{660} at 4 vs
30C, where the A_{660} decreased for all three samples, suggest that PME was
active and was destabilizing the cloud. It also suggests that another influence
was operating at 30C to reduce the cloud loss (HEJ) or increase the A_{660} (MEJ
and SEJ). This other influence could be related to the occurrence of hesperidin
in the juice (Hendrickson and Kesterson 1964). Rothschild and Karsenty (1974)
suggested that a decrease in transmittance observed in shamouti orange juice was
due to the crystallization of hesperidin. Hendrickson and Kesterson (1952) stated

that an increase of evaporator scale (composed of 95-99% hesperidin) was due to increased extraction pressures. It is possible then, that the small decrease in A_{660} for HEJ at 30C was due to a balance between crystallization of increased hesperidin content and the presence of more pulp, rag and peel material from the increased extraction pressure, on which PME could have been more active. Increased extractor pressure has been concluded to lead to an increase of fruit peel material in the juice (Swift 1951; Swift and Veldhuis 1957). Increased rag and peel solids could account for the greater initial A_{660} for the HEJ and the larger decrease in A_{660} through time due to PME activity.
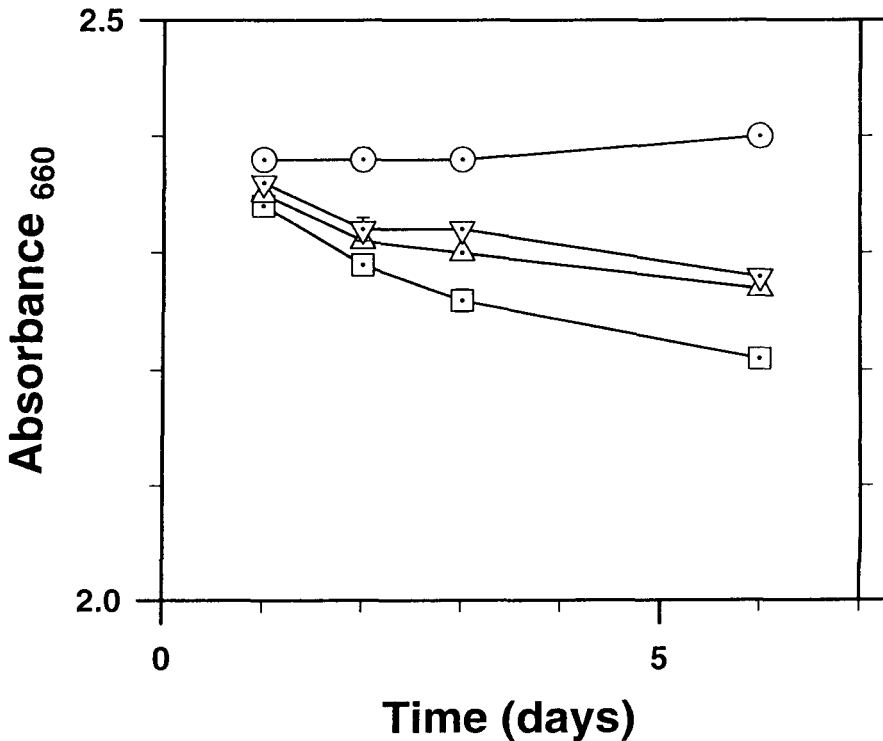

FIG. 5. CLOUD STABILITY AT 4C OF PASTEURIZED, RECONSTITUTED FCOJ TO WHICH PME CONTAINING PROTEIN EXTRACTS FROM SOFT, MEDIUM OR HARD JUICE EXTRACTION HAD BEEN ADDED AT 0.25 UNIT $\cdot$ ML^{-1}
Symbols as in Fig. 3.

Using total salt extracted proteins from each of the juices to add an equal amount of PME activity to pasteurized, reconstituted FCOJ demonstrated that the PME source did have an effect on the cloud stability (Fig. 3). Additionally, the more PME activity added, the more rapidly the juice cloud was destabilized. Unexpectedly, it was the PME extract from the SEJ that destabilized the cloud most rapidly, in contrast to what was observed with the Valencia extracts.

It seems evident that the cloud of Hamlin juice is much different than that of Valencia juice. Not only are the A_{660} values for the Hamlin cloud lower than for Valencia, but, although there is more PME activity estimated in the raw Hamlin juice (from 3 to 4 times as much, see Cameron *et al.* 1999), the Hamlin juice did not clarify over a three week period at 30C. In fact, only the HEJ had a decrease in A_{660} over the sampling period.

These results make it evident that generalizing about cloud behavior and PME content from one sweet orange variety to another is not appropriate. Also, it is obvious that altering the extractor settings does affect the juice cloud properties.

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